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The aerosol stability characteristics of an Egyptian isolate of Rift Valley fever virus (ZH-501 strain) were determined in a static aerosol chamber. Aerosolized particles had a mass median diameter of 4.0 µm. At 30, 55, 80% relative humidity (RH) the biological decay rate was 0.9, 4.1, and 10.1% per min, respectively. The decay rate data tested significantly different ($P < 0.001$) at each RH. The biological half-life values were 6.9 min at 80% RH, 15.8 min at 55%, and 77.0 min at 30% RH. Comparable decay rates were obtained with a strain (SA-51) isolated in South Africa in 1951.

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Airborne Survival of Rift Valley Fever Virus

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ABSTRACT

The aerosol stability characteristics of an Egyptian isolate of Rift Valley fever virus (ZH-501 strain) were determined in a static aerosol chamber. Aerosolized particles had a mass median diameter of 4.0 μm. At 30, 55, 80% relative humidity (RH) the biological decay rate was 0.9, 4.1, and 10.1% per min, respectively. The decay rate data tested significantly different ($P < 0.001$) at each RH. The biological half-life values were 6.9 min at 80% RH, 15.8 min at 55% RH, and 77.0 min at 30% RH. Comparable decay rates were obtained with a strain (SA-51) isolated in South Africa in 1951.

Rift Valley fever (RVF) virus causes a severe febrile disease in several mammalian species including sheep, goats, cattle, and man (5). It occurs naturally only on the African continent. Taxonomically, the virus is classified in the family Bunyaviridae (17), and it cross-reacts serologically with viruses of the phlebotomus fever serogroup (18). Mosquitoes are implicated as the principle vector responsible for transmission of disease in domestic livestock, but their role in the maintenance cycle is unclear (4,6,16). Recent evidence, however, suggests that other means of transmission may be equally important in the infection of humans (8,12). Epidemiological surveys of human disease which occurred in South Africa and Egypt indicated that direct contact with infected animal tissues was a frequent means of human infection. It is also suspected that airborne transmission may occur (8,16).

The first published study on the aerosol properties of RVF virus was by Miller et al. who reported that the aerosolized virus was highly stable and infectious (14). Their data showed that the geometric mean biological decay rate was 2.3% per minute over the range of 50 and 80% relative humidity (RH), and that aerosol stability was not influenced by RH. The purpose of this study was to corroborate their findings and evaluate the potential for airborne transmission of a RVF virus strain recently isolated in Egypt.

The studies were conducted in a maximum containment laboratory using two strains of RVF virus: (i) ZH-501, isolated in Egypt in 1977 from a patient with fatal hemorrhagic fever (13), and (ii) SA-51, isolated in South Africa in 1951 from a naturally infected lamb. The ZH-501 strain had been passed twice in fetal rhesus lung (FRhL) cells and the SA-51 strain had been passed once in mice, three times in sheep, and twice in FRhL cells. A working stock of each strain was prepared by inoculation of Vero cell monolayers maintained in Eagle's

minimal essential medium with Earle's balanced salt solution (EMEME), supplimented with glutamine, and nonessential amino acids, 10% heat-inactivated fetal calf serum, 100 U of penicillin G per ml and 100 µg streptomycin per ml. The monolayers were incubated at 37°C for 48 h, at which time the maintenance medium contained 7.6 to 8.0 $\log_{10}$ plaque-forming units (PFU) per ml. The medium was then collected, centrifuged (1500 x g) for 15 min to remove cellular debris and stored at -70°C.

For each trial an aerosol was generated from 5 ml of virus working stock with an FK-8 atomizer operated at 50 lb/in^2 (gauge) using liquid nitrogen as a propellant (9). The aerosols had a mass median diameter of approximately 4 µm (11) and were disseminated into an environmentally controlled static aerosol chamber having a total volume of 6,200 liters. The chamber is cylirdrical, gastight, and measures approximately 183 cm (6 feet) in diameter by 213 cm (7 feet) in height. The operating principles and design characteristics of the tank have been previously described (19). It has been determined in extensive tests that aerosols generated with the FK-8 atomizer have a physical decay rate in this particular chamber of 1.5% per min (11).

Five replicate one-hour aerosol trials were conducted with the ZH-501 strain at 30, 55, and 80% RH at a constant temperature of 24°C (75°F). Two replicate trials were conducted at each RH with the SA-51 strain. During each trial aerosol samples were collected in duplicate with all-glass impingers calibrated to sample at the rate of 12.5 liters per min (2). Each impinger contained 20 ml of collection fluid consisting of EMEME with 5% heat inactivated fetal calf serum, antibiotics, and antifoam Y-30 emulsion (Dow Corning Corp., Midland, Mich.) at a concentrtion of 1:1000. Aerosols were sampled for five minutes at period midpoints of 4, 32, and 60 min. Contents of the two samplers within each time period were pooled for viral assay. Assays

were performed in duplicate by plaque enumeration using Vero cells (3), and viral titers were calculated as PFU per ml.

Aerosol sampling results were fitted to the simple exponential equation of the form:

$$C_t = C_o e^{-kt}$$

in which C represents the virus concentration in aerosol expressed as percent recovery, t represents aerosol age in minutes, and k represents the linear rate at which the natural logarithm (to base e) of aerosol concentration decreases with aerosol age. From this equation, 100k yields the aerosol decay rate expressed as percent per minute. The biological decay rate was calculated simply as the difference between the total and physical decay rate. The percent virus recovery for each sampling time was the ratio X 100 of the number of PFU recovered to the number of PFU disseminated from the virus working stock at time "0."

The biological decay rate of the ZH-501 strain determined on the basis of five replicate trials was 0.9, 4.1, and 10.1% per minute at relative humidities of 30, 55, and 80%, respectively (Table 1). At each RH, the decay rate is statistically different from the other two, both in terms of slope and elevation ($\underline{P} < 0.001$). These differences are also evident in a plot of percent recovery vs. time shown in Fig. 1. An evaluation of the data from the perspective of biological half-life yielded values of 6.9 min at 80% RH, 15.8 min at 55% RH and 77 min at 30% RH. The total decay rate of the SA-51 strain as determined from results of two replicate trials was 1.3, 2.4, and 6.1% per min at 30, 50, and 80% RH, respectively (Table 1). The decay rates at 55% and 80% RH appear to be slightly lower than those observed for the ZH-501 strain, however,

the difference is not significant. The decay rate of the SA-51 strain increased in relation to an increase in the RH, similar to the increase which occurred with the ZH-501 strain.

For each RH, the percentage recovery of virus at the initial 4-min sampling period exceeded 100%, varying from a geometric mean of 103% for the ZH-501 strain to 265% for the SA-51 strain. It would seem impossible to be able to recover more virus from the aerosol chamber than was present in the virus working stock. This discrepancy may be explained if one assumes viral aggregates were present in the working stock and that the shearing forces of aerosolization dispersed or deagglomerated the clumps of virions. RVF virus replicates by budding into intracytoplasmic vesicles (15), thus providing indirect evidence for the existance of viral clumps. Also, the plaque enumeration technique currently used for virus quantitation will not distinguish between individual virions and virus aggregates; therefore, the procedure could provide a low estimate of the total number of virions present in a particular suspension that contains significant amounts of clumps.

Our results do not agree with the previous findings of Miller et al. who reported there was no difference in the decay rate between 50 and 80% RH (14). The reasons for the conflicting results are not clear. The current technology for viral assay is more sensitive and could account for the variation. It is also important to note that Miller et al. did not conduct trials below 50% RH. In addition, both the suspending fluid and the concentration of protein in the virus working stock used in two studies were different. The suspending fluid is known to be a critical factor in stabilizing the viability of virus in aerosols (1). It should be stressed, however, that in other respects, the results of the two studies were in agreement. In both studies, the virus

was found to be highly stable in aerosol form and the decay rates determined in the range of 50-55% RH were comparable.

The results clearly indicate that the aerosol stability of RVFV is inversely related to RH, i.e., as the RH decreases the aerosol stability increases. A similar inverse relationship was recently reported for Japanese B encephalitis virus (10). It should be pointed out that these studies were performed only in the presence of incandescent lighting and that effect of sunlight was not considered. The importance of the findings in relation to the natural transmission of RVF virus is unknown. Airborne transmission is not thought to play a significant role in the spread of RVF in livestock (5). In humans, however, airborne transmission may occur more frequently than is generally recognized (7,8). Airborne RVF virus is known to be highly infectious and infectious aerosols could be inadvertently generated rather easily from infected animal tissues or fetal membranes which may contain concentrations as high as 10.0 $\log_{10}$ PFU per g. Abattoir workers, veterinarians, and animal handlers are at a particularly high risk.

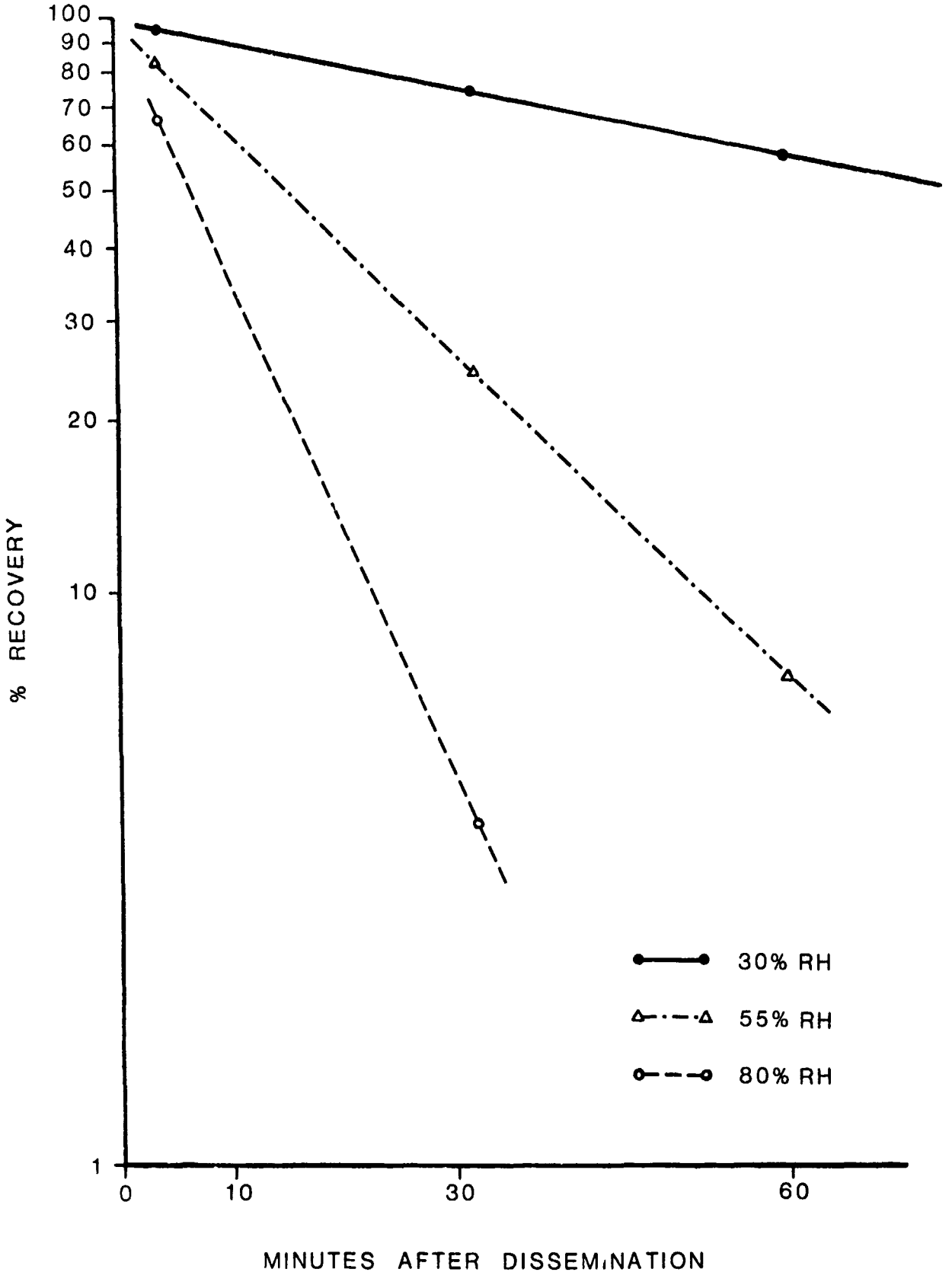
TABLE 1. Biological decay rate and percent recovery of aerosolized Rift Valley fever virus

Viral strain	Relative humidity	Biological decay rate[a] (%/min)	(range)	Biological half-life (minutes)
ZH-501	30%	0.9	(0.3 - 1.5)	77.0
(n = 5 trials)	55%	4.1	(3.4 - 4.5)	15.8
	80%	10.1	(8.6 - 13.3)	6.9
SA-51	30%	1.3	(1.1 - 1.6)	53.3
(n = 2 trials)	55%	2.4	(2.1 - 2.7)	28.9
	80%	6.1	(6.0 - 6.1)	11.4

[a]Geometric mean.

LEGEND

Fig 1. Effect of relative humidity on survival of airborne Rift Valley fever virus (ZH-501 strain) at 24°C. Five ml of virus suspension was disseminated in a 6,200 liter aerosol chamber and air samples were collected at 4, 32, and 60 minutes. Viable virus was identified as PFU per liter of air by plaque assay in Vero cell. Each line is the mean of five replicate experiments.


100
90
80
70
60
50
40
30
20
10
1
% RECOVERY
0
10
30
60
MINUTES AFTER DISSEMINATION
30% RH
55% RH
80% RH

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